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Localization of Two X-Linked Mental Retardation (XLMR) Genes to Xp: MRX37 Gene at Xp22.31–p22.32 and a Putative MRX Gene on Xp22.11–p22.2

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MRX genes of 2 families with X-linked mental retardation (XLMR) were localized by linkage analysis. In family A, the gene was mapped to Xp22.31–p22.32, with significant LOD scores to various Xp22 markers within a distance of 6 Mb between DXS1223 and DXS1224. The MRX gene of this family was designated MRX37. In a mentally retarded female who is a carrier of the MRX37 gene, a random pattern of X inactivation was demonstrated. In family B, a positive LOD score, although not significant (<+2), was found with the marker DXS1202 at Xp22.11–p22.2.
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KEY WORDS: MRX genes, MRX37, Xp22, X inactivation, Fra(X)B

INTRODUCTION

Nonsyndromic or nonspecific mental retardation (MR) is a genetically heterogeneous condition [Neri et al., 1994]. It can be caused by autosomal or X-linked genes, either recessive or dominant. The absence of phenotypic characteristics in individuals with nonspecific MR precludes a combined linkage analysis in the various families. In X-linked MR, the mode of inheritance makes it possible to assign the MR gene to the X chromosome and thus linkage analysis is feasible, provided the families are large enough to give a significant LOD score (of at least +2) [Mulley et al., 1992]. Our aim is to identify families with X-linked MR and to map the relevant genes to provide a means for genetic counseling and join a collaborative effort of mapping X-linked MR (MRX) genes as a first step in their cloning. In the present report, we present the linkage data in 2 families with the apparently XLMR trait.

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The Families

Family A (MRX37). Family A has 6 MR males and one MR female in 2 generations (Fig. 1A). Five of the MR males and the MR female are offspring of 4 healthy sisters and the sixth male is their maternal uncle. In this family, there are 9 normal males.

We examined 3 MR individuals (III-13, III-15, and III-20). Individual III-13 was born prematurely in 1962. During his early years, he had repeated infections in the respiratory tracts and several episodes of convulsions. At puberty, a sexual growth delay was evident and hypogonadism was noted; for that reason, he was followed up by an endocrinologist and received hormone therapy. We first met him when he was 31 years old; his IQ was 52, and he had visnomotor incoordination. On physical examination, his occipito-frontal circumference (OFC) was 54 cm (25–50th centile), his height was 163 cm (3–10th centile), and his weight was 65.4 kg (25–50th centile). He had a female habitus and micropenis. His ears were large; his palpebral fissures were down-slanting; he had narrow frontal area, with sloping forehead, arched palate, and prominent lower maxilla, resulting in an abnormal bite. Individual III-15 was born in 1972. He had convulsions in his first year of life. We first saw him when he was 16 years old. He had mild to moderate mental retardation, a learning disability, and behavior problems and attended a special education program. On physical examination, his OFC was 54 cm (25–50th centile), his height was 153.5 cm (>3rd centile), and his weight was 46 kg (3–10th centile). His ears were large, and he had minor anomalies. There was some facial resemblance between brothers III-13 and III-15; the sexual development of III-15 was normal. Individual III-20 was born in 1973 and was examined at 19 years old; he had moderate mental retardation. On physical examination, his OFC was 56 cm (50th centile), height 166 cm (10–25th centile), and weight 75.5 kg (90th centile). He did not have any minor facial anomalies, and his sexual development was normal. Individual II-5 was severely retarded, and he has been in an institution for years. He was not seen by us. The female III-10 is mildly retarded, but no other details were available.

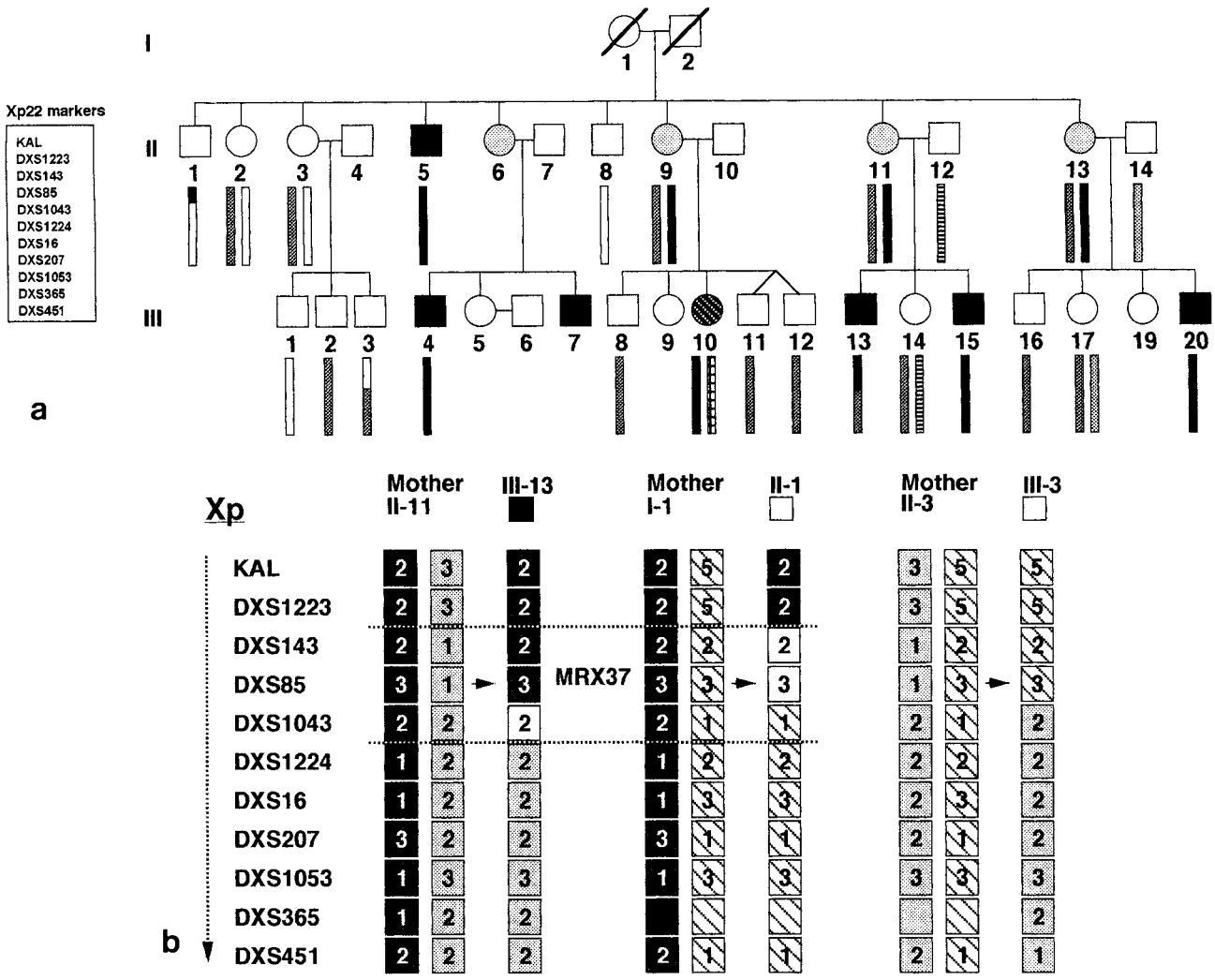

Fig. 1. **a:** Pedigree of family A. Bars represent the various haplotypes at Xp22 (the polymorphic markers that constitute the haplotypes are listed in the box). Solid box = MR male, gray circle = obligate carrier, striped circle = MR female (was not included in the linkage analysis). **b:** Recombinant chromosomes at Xp of family A members.

Family B. Family B has 4 mentally retarded males: 3 are brothers and the fourth is the son of a maternal aunt. In addition, they have 2 brothers and 4 sisters, all of them healthy (Fig. 2A). There is no detailed clinical information of the MR individuals of this family.

Fra(X)A was ruled out in both families by cytogenetic and molecular analyses.

MATERIALS AND METHODS

DNA was prepared from whole blood according to standard techniques. The polymorphic markers were of the microsatellite type, most of them being (CA)n dinucleotide repeats. The primer sequences were drawn from the Genome Data Base (GDB) and from the Généthon linkage map [Gyapay et al., 1994]. The analysis of the polymorphic markers was based on the polymerase chain reaction (PCR). PCR products were separated on a denatured polyacrylamide gel and detected by silver staining [Lerer et al., 1994].

Two-point LOD scores were obtained with the computer program LIPED [Ott, 1976]. We assumed a simple recessive X-linked model that was fully penetrant in males; therefore, we did not include the MR female from family A (III-10) in the linkage analysis.

The pattern of X inactivation was studied by the method described by Allen et al. [1992], except that the PCR products were detected by silver staining as described elsewhere [Abeliovich et al., 1995].

Fra(X)B analysis was done on peripheral blood from the patients and 2 normal controls. Blood was cultured in RPMI 1640 medium according to standard techniques [Gosden et al., 1992]. Fra(X)B was induced by 0.2 mM aphidicolin (Sigma) that was introduced into the culture medium 24 hr before harvesting [Glover et al., 1984]. GTG banding was performed according to Benn and Perle [1992].

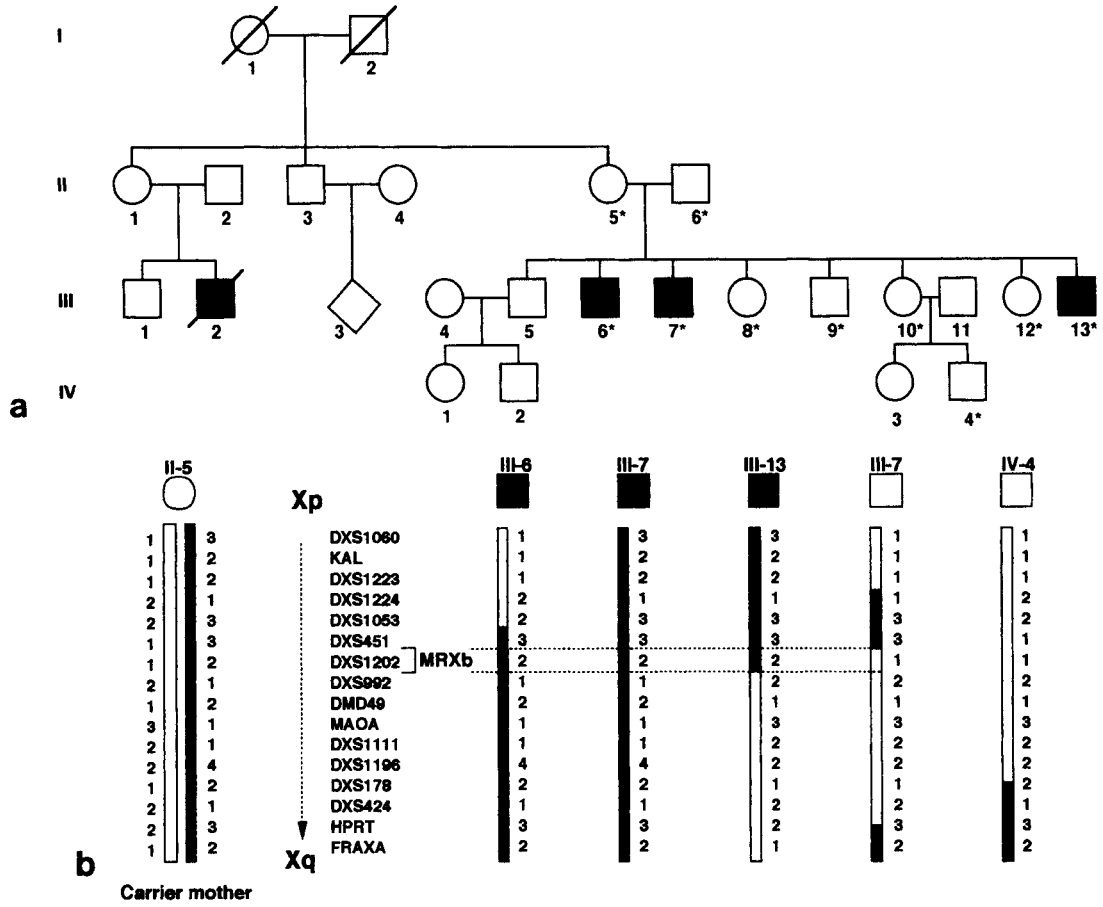

Fig. 2. **a:** Pedigree of family B (* indicates individuals who participated in the linkage analysis). **b:** Recombinant chromosomes of individuals of family B.

RESULTS

Linkage Analysis

We set up a panel of X-linked polymorphic microsatellite markers, with average distances of 20–30 cM along the X chromosome. The physical map of the X-chromosome markers is based on that by Willard et al. [1994], and the genetic distances between the polymorphic markers are set according to Gyapay et al. [1994].

Family A (MRX37). In the preliminary analysis, markers of the long arm, DXS1111 and DXS178, gave negative LOD scores, and markers of the short arm, MAOA, DMD49, DXS451, and DXS365, gave positive LOD scores with increased values as the marker was more distal on the Xp (Table I). This was a hint that the MRX gene of family A may reside in the distal part of the short arm, pointing to Xp22 as the possible location. We then concentrated on markers of the distal Xp; all of them gave positive and significant LOD scores, allowing the mapping of the MRX gene to this region (Table I). According to the θ values at Z_{max}, the MRX gene should be 10 cM from DXS365 and 5 cM from DXS1053, DXS207, DXS1224, DXS16, KAl, and DXS1223; markers DXS1043, DXS143, and DXS85 gave a Z_{max} within 0 cM. In the physical map, the best possible location was between DXS1224 and KAL at Xp22.31–22.32, within a distance of 6 Mb (Fig. 3). The significant LOD scores that were obtained with the MRX gene of family A allowed the marker to be designated as MRX37.

The haplotypes at distal Xp of the relatives were constructed (Fig. 1B). Recombinant chromosomes were apparent in 3 individuals: II-1, III-3, and III-13. The recombinant chromosomes of II-1 and III-13 were a result of a recombination between the normal and the MRX-bearing chromosomes. In III-3, the recombinant chromosome was a result of a recombination between 2 normal chromosomes (Fig. 1B). This individual had a recombinant chromosome in which the markers KAL, DXS1223, DXS143, and DXS85 were of one homologes of the X chromosome and DXS1043 was of the other one. Based on this recombination, we concluded that the simplest interpretation would be that the position of DXS85 is distal to DXS1043. In individual II-1, the recombination occurred between loci DXS1223 and DXS1043 (the markers between them were not informative). Because II-1 is healthy, the MRX37 gene must be proximal to DXS1223; in individual III-13, the re-

TABLE I. Pairwise LOD Scores Between MRX37 Gene (Family A) and Markers on the X Chromosome

Xp	Locus	θ 0.00	0.001	0.05	0.1	0.2	0.3	0.4	Z_{max}[a]	θ_{max}
	KAL	−11.1	1.58	2.94	2.88	2.39	1.69	0.94	**2.94**	0.05
	DXS1223	−11.1	1.75	3.10	3.03	2.51	1.77	0.88	**3.10**	0.05
	DXS143	2.34	2.33	2.16	1.97	1.58	1.13	0.61	**2.34**	0.00
	DXS85	2.46	2.46	2.27	2.08	1.66	1.19	0.64	**2.46**	0.00
	DXS1043	1.88	1.87	1.70	1.52	1.14	0.72	0.27	1.88	0.00
	DXS1224	0.46	1.49	2.83	2.78	2.32	1.64	0.79	**2.83**	0.05
	DXS16	−0.1	1.02	2.38	2.37	1.99	1.42	0.67	**2.38**	0.05
	DXS207	0.89	1.92	3.22	3.13	2.6	1.8	0.9	**3.22**	0.05
	DXS1053	0.46	1.49	2.83	2.78	2.32	1.64	0.79	**2.83**	0.05
	DXS365	−0.3	0.72	2.10	2.11	1.79	1.28	0.62	**2.11**	0.1
	DXS451	1.88	1.87	1.70	1.52	1.13	0.71	0.26	1.88	0.00
	DMD49	−99.	−4.2	0.38	0.97	1.20	0.97	0.51	1.20	0.2
	MAOA	−99.	−7.89	−1.4	−0.4	0.18	0.32	0.23	0.32	0.3
	DXS1111	−12.	−11.	−3.9	−2.5	−1.2	−0.5	−0.1	0.00	0.5
	DXS178	−8.1	−6.5	−3.1	−1.9	−0.87	−0.4	−0.1	0.00	0.1

Xq

[a]Numbers in boldface indicate significant Lod scores.

combination occurred between DXS85 and DXS1224 (DXS1043 was not informative), thus the gene is distal to DXS1224.

The conclusion from the 2 recombinant chromosomes was that the MRX37 gene lies between DXS1224 and DXS1223 within a genetic distance of 8 cM. A similar conclusion was obtained by linkage data. Additional markers between them might narrow the map position.

Family B. In this family, the only marker that gave a positive LOD score (0.852) was DXS1202, which is mapped to Xp22.1 (Table II). This is the maximum LOD score expected for this family. The size of the family and the number of individuals who participated in the study did not allow us to obtain a significant LOD score. Negative and excluding data (with exclusion limits from 0.1 cM to <5 cM) were obtained for all the markers.

Haplotypes were constructed for the markers along the X chromosome, and we looked for a common and exclusive allele to the MR individuals. In locus DXS1202, there was an allele common to all the affected males, whereas the normal males had other alleles (Fig. 2B). According to individual III-11, the gene might be distal to DXS992; according to III-5, it is proximal to DXS451. Markers DXS992 and DXS451 are mapped to Xp22.11–22.2 at a distance of 8 Mb on the physical map. However, because we did not get a signicant LOD score (>+2) for any of the markers, there is doubt about our original assumption of an X-linked gene in this family. We urged the family to provide blood samples of more informative individuals, such as female II-1 and males II-3, III-1, and III-5, to increase the LOD score to a significant level.

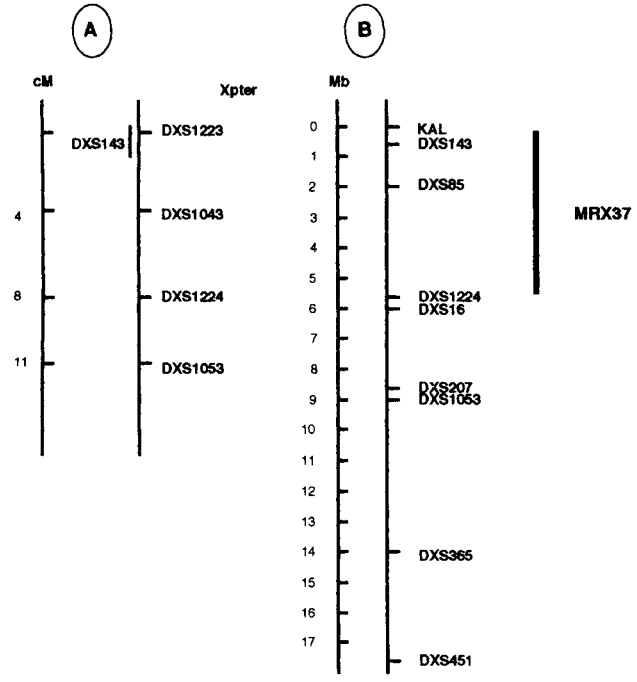

Fig. 3. The localization of the MRX37 gene on the physical [Willard et al., 1994] and genetic [Gyapay et al., 1994] maps of Xp22.

Expression of Fra(X)B in Family A

The locus of the MRX37 gene in family A was mapped to Xp22.31–22.32, to the same region of a common fragile site FRA(X)B [Sutherland, 1991]. It was tempting to speculate that this fragile site might be involved in the MRX gene in such a way that would interrupt the gene if it were abnormally expressed.

Fra(X)B was induced by aphidicoline in lymphocytes of 2 MR individuals. Eight percent of the cells expressed Fra(X)B in individual III-13, 6% in III-15, and 6% and 10% in 2 normal controls. There was no difference in the expression of the FRA(X)B in the affected individuals and the controls: $\chi^2 = 1.578$ ($0.5 < P < 0.75$).

X Inactivation in the MR Female of Family A

Haplotype analysis at Xp22 of family A's members allowed us to determine the carrier status of the females in this family. Female III-10 was found to be a carrier of the MRX37 gene, and she was also the mentally re-

TABLE II. Pairwise LOD Scores Between MRX Gene (Family B) and Markers on the X Chromosome

		θ								
Xp	Locus	0.00	0.001	0.05	0.1	0.2	0.3	0.4	Z_{max}	θ_{max}
	DXS1060	−2.8	−1.8	−0.2	−0.04	0.05	0.03	−0.004	0.05	0.2
	DXS1223	−2.8	−1.8	−0.2	−0.04	0.05	0.03	−0.004	0.05	0.2
	DXS1224	−6.6	−4.7	−1.4	−0.91	−0.4	−0.1	−0.37	0.00	0.5
	DXS1053	−6.8	−5.1	−1.7	−1.1	−0.5	−0.2	−0.06	0.00	0.5
	DXS451	−6.8	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DXS1202	0.85	0.85	0.77	0.68	0.49	0.29	0.092	0.852	0.00
	DXS992	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DMD49	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	MAOA	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DXS1111	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DXS1196	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DXS178	−3.1	−2.1	−0.5	−0.2	−0.1	−0.06	−0.03	0.00	0.5
	DXS424	−6.8	−4.5	−1.2	−0.7	−0.2	−0.11	−0.03	0.00	0.5
	HPRT	−6.6	−5.0	−1.7	−1.1	−0.5	−0.22	−0.06	0.00	0.5
	FRAXAC	−6.6	−4.6	−1.4	−0.8	−0.3	−0.14	−0.03	0.00	0.5
Xq										

tarded one. We analyzed her DNA sample further for the pattern of X inactivation in the assumption that she might be retarded due to nonrandom X inactivation. We analyzed the methylation status of the CpG island in the 5′ region of the androgen receptor gene [Allen et al., 1992]. The results are presented in Fig. 4 with those of 2 female controls who were heterozygous for the length of the trinucleotide (CAG)n: 1 control had a nonrandom pattern and the other had a random pattern of the X chromosome. In patient III-10, the smaller allele was better amplified and was therefore darker. However, the ratio between the intensity of the 2 alleles was the same, with and without HpaII digestion prior to PCR, indicating a random X inactivation.

DISCUSSION

We analyzed two families with multiple MR males, suggesting segregation of a MRX gene. The phenotype of the MR individuals in family A was variable and inconsistent and did not allow us to define this family as syndromic or as nonspecific. Individual III-13 had hypogonadism and delayed sexual development, III-15 had relatively short stature, and III-20 was obese. We prefer to define this family as nonspecific (?) XMR.

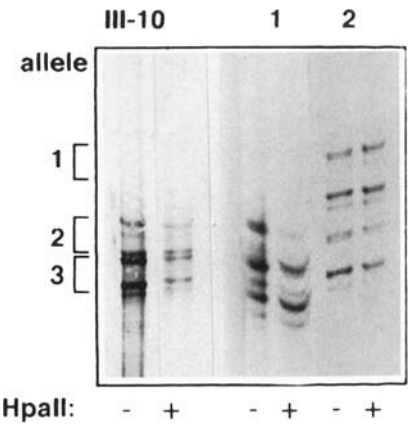

Fig. 4. X inactivation pattern of female III-10 of family A and 2 female controls. **1:** Nonrandom X inactivation. **2:** Random X inactivation.

Family A met the criteria of Mulley et al. [1992] because significant LOD scores (>+2) were obtained for several X-linked markers, and we could assign the MRX37 gene to Xp22.31–22.32. Family B was not large enough to yield a significant LOD score, and the gene was tentatively assigned to Xp22.11–22.2 by identifying nonoverlapping regions between the MR and the normal males. Furthermore, the linkage data with markers along the X chromosome yielded a negative or zero LOD score, which excluded other regions on the X chromosome as a possible location of the putative XMR gene.

Assuming that the gene is indeed X linked in family B, the regional mapping of the MRX genes indicates that there are at least 2 different MRX genes in Xp. The MRX37 of family A may be the same as the MRX24 gene [Martinez et al., 1995] that was mapped 3 cM proximal to DXS85. The MRX gene of family B is in the same region as MRX2, and it might overlap the MRX 19,21 and 13 loci [Neri et al., 1994].

The site of MRX gene of family A was at Xp22.31, a site of the common fragile site Fra(X)B. Although the common fragile sites are not known to be associated with diseases, we analyzed Fra(X)B to rule out the possibility that, due to an "overexpression" of this fragile site, it renders this site to breakage. Our results did not support this possibility.

In family A, there were 5 carrier females: 4 were healthy, whereas 1, individual III-10, was mentally retarded. MR females in families with an MRX gene, which is considered to be an X-linked recessive gene, have been described by others [Kerr et al., 1992; Donnelly et al., 1994]. There are 3 possible explanations for the MR females in MRX families: (1) the MRX is a dominant gene with incomplete penetrance in females, (2) the MRX gene is recessive and is expressed in females due to nonrandom X inactivation, and (3) the MR state

in the female is unrelated to the familial MRX gene. Partial penetrance in families like family A is not a likely explanation because only 1 in 5 females was affected. The MR female of family A (III-10) proved to be a carrier of the MRX gene, and she had a random pattern of X inactivation in her lymphocyte DNA. It is possible that in other tissues such as in the brain, there is a different pattern of X inactivation that may explain the MR. It is also possible that, although she is a carrier of an MRX gene, her mental state is unrelated to the MRX gene.

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